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(71)Applicant : SEITAI KAGAKU KENKYUSHO:KK

(72)Inventor : SHIGEMATSU AKIYO
SUZUKI SATOSHI
KABURAGI TAKAYUKI
SUEYOSHI TORU

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(54) STORING OF TISSUE OF ORGANISM

(57)Abstract:
PURPOSE: To provide a method for storing the tissue composing the organ of an organism so as to maintain the characteristics and functions of the tissue at a cell level.
CONSTITUTION: This method for storing the tissue of the organism comprises exchanging blood in the organ of an organism with the first carbohydrate solution not causing the hemolysis of the blood, further exchanging the first carbohydrate solution with the second carbohydrate solution containing an organic solvent to prevent the generation of ice and solute crystals in the tissue, cooling the treated tissue to the temperature of liquid nitrogen, and subsequently storing the frozen tissue. The tissue specimen is thrust with the aggregation of metal needles to uniformly cool the tissue specimen or uniformly and rapidly thaw the frozen tissue specimen.

[Claim(s)]
[Claim 1] Inject perfusate into the blood vessel which passes to the organ of an animal, and the blood in said organ is permuted by said perfusate. In the living thing organization store method which said a part of organ [at least] excised from said animal is cooled, and temperature is reduced to liquid nitrogen temperature, and saves the organization of said organ The first sugar liquid which does not produce hemolysis substantially as the first perfusate is injected into said blood vessel. In the blood vessel which permutes the blood in the blood vessel in said organ with this first sugar liquid, and passes subsequently to said organ, as the second perfusate The sugar which does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], The living thing organization store method which mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and pours in the second sugar liquid containing the organic solvent which does not produce a crystal substantially, permutes said first sugar liquid in said organ with said second sugar liquid, and is characterized by cooling to liquid nitrogen temperature after that.
[Claim 2] The living thing organization store method of claim 1 in which said second sugar liquid contains mannite as said sugar.
[Claim 3] The living thing organization store method of claim 1 in which said second sugar liquid contains an inulin as said sugar.
[Claim 4] The living thing organization store method of claims 1, 2, or 3 said whose organic solvent is dimethyl sulfoxide or a glycerol.
[Claim 5] claim 1 whose temperature of said second sugar liquid is 0 degree C or less thru/or 4 -- one of living thing organization store methods.
[Claim 6] claim 1 which excises all or the predetermined part of said organs from said animal after said blood permutation with said first sugar liquid thru/or 5 -- one of living thing organization store methods.
[Claim 7] claim 1 in which said first sugar liquid contains a glucose thru/or 6 -- one of living thing organization store methods.
[Claim 8] claim 1 in which said first sugar liquid contains the matter which has monoamine oxidase inhibitory action thru/or 7 -- one of living thing organization store methods.
[Claim 9] claim 1 said whose animal is mammalian thru/or 8 -- one of living thing organization store methods.
[Claim 10] Inject perfusate into the blood vessel which passes to the organ of an animal, and the blood in said organ is permuted by said perfusate. In the living thing organization store method which said a part of organ [at least] excised from said animal is cooled, and temperature is reduced to liquid nitrogen temperature, and saves the organization of said organ Intercept the peripheral nerve of the field containing said organ, and the first isotonicity sugar liquid which does not produce hemolysis substantially as the first perfusate in said blood vessel is poured in. In the blood vessel which permutes the blood in the blood vessel in said organ with this first sugar liquid, and passes subsequently to said organ, as the second perfusate The sugar which does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen

temperature, and], The living thing organization store method which mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and pours in the second sugar liquid containing the organic solvent which does not produce a crystal substantially, permutes said first sugar liquid in said organ with said second sugar liquid, and is characterized by cooling to liquid nitrogen temperature after that.
[Claim 11] The living thing organization store method of claim 10 in which said second sugar liquid contains mannite as said sugar.
[Claim 12] The living thing organization store method of claims 10 or 11 said whose organic solvent is dimethyl sulfoxide or a glycerol.
[Claim 13] claim 10 whose temperature of said second sugar liquid is 0 degree C or less thru/or 12 -- one of living thing organization store methods.
[Claim 14] claim 10 which excises all or the predetermined part of said organs from said animal after said blood permutation with said first sugar liquid thru/or 13 -- one of living thing organization store methods.
[Claim 15] claim 10 in which said first sugar liquid contains a glucose thru/or 14 -- one of living thing organization store methods.
[Claim 16] claim 10 which intercepts a peripheral nerve by introducing a peripheral nerve cutoff agent into the inside of the blood vessel of said animal, or intraperitoneal thru/or 15 -- one of living thing organization store methods.
[Claim 17] claim 10 which is the matter with which said peripheral nerve cutoff agent has monoamine oxidase inhibitory action thru/or 16 -- one of living thing organization store methods.
[Claim 18] claim 10 said whose animal is mammalian thru/or 17 -- one of living thing organization store methods.
[Claim 19] In the living thing organization store method which a part of organ [at least] excised from the animal is cooled, and temperature is reduced to liquid nitrogen temperature, and is saved The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth as an organization sample is run through with said a part of excised organ [at least]. The living thing organization store method which said sample with which this needle aggregate was run through is gradually cooled at the rate of predetermined, and temperature is further reduced to liquid nitrogen temperature, and is characterized by saving at liquid nitrogen temperature.
[Claim 20] Said predetermined rate is a living thing organization store method of claim 19 which is the average of less than 1.3 degrees C/m to -80 degrees C.
[Claim 21] In the living thing organization store method which a part of organ [at least] excised from the animal is cooled, and temperature is reduced to liquid nitrogen temperature, and is saved The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth as an organization sample is run through with said a part of excised organ [at least]. In case cool gradually said sample with which this needle aggregate was run through at the rate of predetermined, temperature is further reduced to liquid nitrogen temperature, it saves at liquid nitrogen temperature and preservation is ended, in order to revive the bioactive of said organization sample, The living thing organization store method characterized by making temperature of the plinth of said needle aggregate into 20 degrees C or more into the time amount which does not exceed 4 minutes.
[Claim 22] The living thing organization store method of claim 21 which makes

temperature of said plinth 20 degrees C or more within 3 minutes in case preservation is ended.

[Claim 23] In the living thing organization store method which perfusate is injected into the blood vessel which passes to the organ of an animal, the blood in said organ is permuted by said perfusate, said a part of organ [at least] excised from said animal is cooled, and temperature is reduced to liquid nitrogen temperature, and is saved The first sugar liquid which does not produce hemolysis substantially as the first perfusate is injected into said blood vessel. In the blood vessel which permutes the blood in the blood vessel in said organ with this first sugar liquid, and passes subsequently to said organ, as the second perfusate The sugar which does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is poured in. Said second sugar liquid permutes said first sugar liquid in said organ, and said a part of organ [at least] excised from said animal is made into an organization sample. The living thing organization store method which the organization sample with which ran through with the needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth, and this needle aggregate was run through is gradually cooled at the rate of predetermined, and temperature is further reduced to liquid nitrogen temperature, and is characterized by saving at liquid nitrogen temperature.

[Claim 24] The living thing organization store method of claim 23 said whose organic solvent is dimethyl sulfoxide or a glycerol.

[Claim 25] The living thing organization store method of claims 23 or 24 in which said second sugar liquid contains mannite as said sugar.

[Claim 26] The living thing organization store method of claims 23 or 24 in which said second sugar liquid contains an inulin as said sugar.

[Claim 27] claim 23 in which said first sugar liquid contains mannite as main sugar thru/or 26 -- one of living thing organization store methods.

[Claim 28] claim 23 in which said first sugar liquid contains a glucose thru/or 26 -- one of living thing organization store methods.

[Claim 29] claim 23 said whose predetermined rate is the average of less than 1.3 degrees C/m to -80 degrees C thru/or 28 -- one of living thing organization store methods.

[Claim 30] claim 23 which excises all or the predetermined part of said organs from said animal after said blood permutation with said first sugar liquid thru/or 29 -- one of living thing organization store methods.

[Claim 31] The first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to the organ of an animal. The sugar which permutes the blood in said organ with said first sugar liquid, excises all or the predetermined part of said organs between this permutation or after a permutation, and subsequently does not produce a crystal substantially [a water solution does not carry out phase separation to said blood vessel in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is poured in. Said a part of organ [at least] which permuted said first sugar liquid in said organ with said second sugar liquid, and was permuted with this second

sugar liquid as an organization sample The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through. Said sample with which said needle aggregate was run through is gradually cooled at the rate of predetermined. A part of [at least] temperature of said needle aggregate in the time amount which does not exceed 4 minutes in case temperature is furthermore reduced to liquid nitrogen temperature, it saves at liquid nitrogen temperature and preservation is ended as 20 degrees C or more The living thing organization store method which the temperature of said sample is raised more than the freezing point of said second sugar liquid, and consists of drawing out said sample from said needle aggregate.
[Claim 32] The living thing organization store method of claim 31 said whose organic solvent is dimethyl sulfoxide or a glycerol.
[Claim 33] The living thing organization store method of claims 31 or 32 in which said second sugar liquid contains mannite as said sugar.
[Claim 34] The living thing organization store method of claims 31 or 32 in which said second sugar liquid contains an inulin as said sugar.
[Claim 35] claim 31 whose predetermined rate of said cooling is the average of less than 1.3 degrees C/m to -80 degrees C thru/or 34 -- one of living thing organization store methods.
[Claim 36] claim 31 in which said first sugar liquid contains mannite as main sugar thru/or 35 -- one of living thing organization store methods.
[Claim 37] claim 31 in which said first sugar liquid contains a glucose thru/or 35 -- one of living thing organization store methods.
[Claim 38] claim 31 whose time amount which does not exceed said 4 minutes is less than 3 minutes thru/or 37 -- one of living thing organization store methods.
[Claim 39] The living thing organization store method of claim 38 which makes temperature of the plinth of said needle aggregate 20 degrees C or more within 3 minutes in case preservation is ended.
[Claim 40] The first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to the organ of an animal. The sugar which permutes the blood in said organ with said first sugar liquid, and subsequently does not produce a crystal substantially [a water solution does not carry out phase separation to said blood vessel in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is poured in. Excise all or the predetermined part of said organs which permuted said first sugar liquid in said organ with said second sugar liquid, and were permuted with this second sugar liquid from said animal, and this is made into an organization sample. The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through. Said sample with which said needle aggregate was run through is gradually cooled at the rate of predetermined. In case temperature is furthermore reduced to liquid nitrogen temperature, it saves at liquid nitrogen temperature and preservation is ended, a part of [at least] temperature of said needle aggregate in predetermined time amount as 20 degrees C or more The living thing organization store method which the temperature of said sample is raised more than the freezing point of said second sugar liquid, and consists of drawing out said sample from said needle aggregate.

[Claim 41] The living thing organization store method of claim 40 said whose organic solvent is dimethyl sulfoxide or a glycerol.
[Claim 42] The living thing organization store method of claims 40 or 41 in which said second sugar liquid contains mannite as said sugar.
[Claim 43] The living thing organization store method of claims 40 or 41 which makes temperature of said second sugar liquid 0 degree C or less.
[Claim 44] claim 40 which is that from which said first sugar liquid does not produce a crystal substantially in liquid nitrogen temperature thru/or 43 -- one of living thing organization store methods.
[Claim 45] The living thing organization store method of claim 44 in which said first sugar liquid contains mannite as main sugar.
[Claim 46] The living thing organization store method of claim 44 in which said first sugar liquid contains a glucose.
[Claim 47] The predetermined rate of said cooling is claim 40 thru/or the living thing organization store method of 46 which is the average of less than 1.3 degrees C/m to -80 degrees C.
[Claim 48] claim 40 which makes temperature of the plinth of said needle aggregate 20 degrees C or more within 3 minutes in case preservation is ended thru/or 47 -- one of living thing organization store methods.
[Claim 49] The first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to the organ of an animal. The sugar which permutes the blood in said organ with said first sugar liquid, and subsequently does not produce a crystal substantially [a water solution does not carry out phase separation to said blood vessel in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is poured in. All or the predetermined part of said organs which permuted said first sugar liquid in said organ with said second sugar liquid, and were permuted with this second sugar liquid as an organization sample The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through. Said sample with which said needle aggregate was run through is gradually cooled at the rate of predetermined. The living thing organization store method which temperature is furthermore reduced to liquid nitrogen temperature, and it saves at liquid nitrogen temperature, the temperature of said sample is raised in predetermined time amount more than the freezing point of said second sugar liquid in case preservation is ended, and consists of drawing out said sample from said needle aggregate.
[Claim 50] The living thing organization store method of claim 49 said whose organic solvent is dimethyl sulfoxide or a glycerol.
[Claim 51] The living thing organization store method of claims 49 or 50 in which said second sugar liquid contains mannite as said sugar.
[Claim 52] claim 49 whose predetermined rate of said cooling is the average of less than 1.3 degrees C/m to -80 degrees C thru/or 51 -- one of living thing organization store methods.
[Claim 53] claim 49 in which said first sugar liquid contains a glucose thru/or 52 -- one of living thing organization store methods.
[Claim 54] claim 49 which raises the temperature of said sample within 4 minutes at -5

degrees C or more 5 degrees C or less in case preservation is ended thru/or 53 -- one of living thing organization store methods.
[Claim 55] The living thing organization store method of claim 54 which raises -5 degrees C or more 5 degrees C or less within 4 minutes, and raises the temperature of said sample at 25 degrees C or more at a rate with an average of 4 degrees C [or less]/m in case preservation is ended.
[Claim 56] Inject perfusate into the blood vessel which passes to the organ of an animal, and the blood in said organ is permuted by said perfusate. In the living thing organization store method which said a part of organ [at least] excised from said animal is cooled, and temperature is reduced to liquid nitrogen temperature, and saves some organizations [at least] of said organ Before pouring in said perfusate, intercept the peripheral nerve of the field which contains said organ of said animal beforehand, and the first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to said organ. The sugar which permutes the blood in said organ with said first sugar liquid, and does not produce a crystal substantially [excise said organ from said animal after that, and a water solution does not carry out phase separation under this permutation or to the blood vessel which passes subsequently to said organ in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is poured in. Said a part of organ [at least] which permuted said first sugar liquid in said organ with said second sugar liquid, and was permuted with this second sugar liquid as an organization sample The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through. Said sample with which said needle aggregate was run through is gradually cooled at the rate of predetermined. The living thing organization store method which temperature is furthermore reduced to liquid nitrogen temperature, and it saves at liquid nitrogen temperature, the temperature of said sample is raised in the time amount which does not exceed 4 minutes more than the freezing point of said second sugar liquid in case preservation is ended, and consists of drawing out said sample from said needle aggregate.
[Claim 57] The living thing organization store method of claim 56 said whose organic solvent is dimethyl sulfoxide or a glycerol.
[Claim 58] The living thing organization store method of claims 56 or 57 in which said second sugar liquid contains mannite as said sugar.
[Claim 59] claim 56 in which said first sugar liquid contains a glucose thru/or 58 -- one of living thing organization store methods.
[Claim 60] claim 56 whose predetermined rate of said cooling is the average of less than 1.3 degrees C/m to -80 degrees C thru/or 59 -- one of living thing organization store methods.
[Claim 61] claim 56 which raises the temperature of said sample within 4 minutes at -5 degrees C or more 5 degrees C or less in case preservation is ended thru/or 60 -- one of living thing organization store methods.
[Claim 62] The living thing organization store method of claim 61 which raises -5 degrees C or more 5 degrees C or less within 4 minutes, and raises the temperature of said sample at 25 degrees C or more at a rate with an average of 4 degrees C [or less]/m in case preservation is ended.

[Claim 63] In the living thing organization preservation playback approach of cooling a part of organ [at least] excised from the animal, thawing said organization sample after reducing temperature and saving it to liquid nitrogen temperature, and recovering bioactive Run through the needle aggregate which consists of two or more metal needles fixed in parallel with a metal plinth with all or the predetermined part of said excised organs, and it cools. In case it saves as an organization sample at liquid nitrogen temperature and preservation is ended, in order to revive the bioactive of said organization sample, Said organization sample is thawed using [warm an end, even if there are few said metal needles, and] temperature of said organization sample as -5 degrees C or more 5 degrees C or less within 4 minutes. The living thing organization preservation playback approach which said organization sample is sampled from said needle aggregate, and the temperature of the account organization sample of defrosting back to front is raised at 25 degrees C or more at a rate with an average of 4 degrees C [or less]/m, and is characterized by recovering the bioactive of said organization sample.
[Claim 64] Inject perfusate into the blood vessel which passes to the organ of an animal, and the blood in said organ is permuted by said perfusate. In the living thing organization preservation playback approach of thawing said organization sample and recovering bioactive after cooling said a part of organ [at least] excised from said animal, reducing temperature to liquid nitrogen temperature and saving the organization of said organ Run through the needle aggregate which consists of two or more metal needles fixed in parallel with a metal plinth with all or the predetermined part of said excised organs, and it cools. In case it saves as an organization sample at liquid nitrogen temperature and preservation is ended, an end is warmed even if there are few said metal needles. Said organization sample is thawed using temperature of said organization sample as -5 degrees C or more 5 degrees C or less within 4 minutes. The living thing organization preservation playback approach which the temperature of said organization sample is raised at 25 degrees C or more after it samples said organization sample and 5 minutes pass at least after defrosting since said needle aggregate, and is characterized by recovering the bioactive of said organization sample.
[Claim 65] The living thing organization preservation playback approach of claim 64 of recovering the bioactive of said organization sample by carrying out perfusion of the buffer solution which contains sugar and a serum protein in the blood vessel which passes to said organization sample while raising the temperature after defrosting to 25 degrees C.
[Claim 66] The living thing organization preservation playback approach of claim 65 that said buffer solution contains dibutyryl cyclic AMP.

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the cold storage approach and the preservation playback approach of the living thing organization which can maintain the property and function of a cell which constitute a living thing organization store method, especially an organization.
[0002]
[Description of the Prior Art] Since cells, such as microorganisms, such as bacteria, and

an ovum, a sperm, a cultured cell, are saved so that activity may be maintained 50% or more, a cell is gradually cooled with the speed around 1 degree C/m, it saves around -200 degrees C, and the approach of dissolving quickly (defrosting) is learned at the time of preservation termination. Under the present circumstances, in order to prevent destruction by freezing of a cell, a cell is placed into 10% glycerol or 5 - 10% dimethyl sulfoxide water solution.

[0003]

[Problem(s) to be Solved by the Invention] The cell according to individuals, such as a microorganism, an ovum, and a sperm, can be comparatively saved by the above-mentioned approach for a long period of time. However, when such liquid was injected into organs, such as the organ of an animal, for example, liver, and the kidney, the property of the cell of an organization and the loss of a function which were saved were remarkable.

[0004] The purpose of this invention is offering the approach of saving the organization which constitutes the organ of a living thing so that the property's and function's may be maintained on cell level. Moreover, the purpose of this invention is offering the approach of recovering bioactive, after saving the organization which constitutes the organ of a living thing so that the property and function may be maintained on cell level.

[0005]

[Means for Solving the Problem] In order that the living thing organization store method of this invention may attain this purpose, the first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to the predetermined organ of an animal. The sugar which permutes the blood in an organ with the first sugar liquid, and does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is injected into a blood vessel. The second sugar liquid permutes the first sugar liquid in a blood vessel, and all or the predetermined part of organs is excised. The needle aggregate which consists of the metal needle of a large number fixed in parallel is run through with the sample of this excised organ. In case cool gradually at the rate of predetermined, temperature is further reduced to liquid nitrogen temperature, the sample with which the needle aggregate was run through is saved at liquid nitrogen temperature and preservation is ended, a part of [at least] temperature of the needle aggregate is quickly made into 20 degrees C or more. The temperature of a sample is promptly raised to more than the freezing point of the second sugar liquid, and it consists of drawing out a sample from the needle aggregate. This invention is applicable not only to organs, such as an organ of an animal, but connective tissue, such as muscles.

[0006] Instead of excising an organization sample after a permutation (henceforth perfusion) with the first [of blood], and second sugar liquid, all or the predetermined part of organs may be excised from an animal between the perfusion before perfusion. A predetermined part is one of the hepatic lobes in liver, or two. In order to perform perfusion or excision, it is necessary to conduct an operation for an animal. When performing perfusion after excision, some organizations may be further excised as a sample after perfusion.

[0007] Before undergoing an operation, it is desirable to intercept the peripheral nerve

near [used as especially an object] the organ of an animal because of maintenance of an organization function. Cutoff of the peripheral nerve near an object organ can be attained by pouring into the inside of a blood vessel, or intraperitoneal the matter which has monoamine oxidase (following, MAO, and brief sketch) inhibitory action. There are for example, N-amino alkyl phenothiazin system compound, N-amino alkyl JIBENZOAZA cycloheptadiene system compound, an isonicotinic acid hydrazide system compound, and a propargyl amine derivative in MAO inhibitor applicable to this invention. For example, they are chlorpromazine, a promethazine, imipramine, the iproniazid, pargyline, etc. MAO inhibitor may be added also in perfusate.

[0008] Perfusion usually stabs with a detention needle (hollow) the blood vessel which passes to an organ, and is performed. A flow rate is chosen according to the class of organ, the class of blood vessel, and a size. The permutation with the first and second sugar liquid is enough performed so that blood and the first sugar liquid may not remain substantially, respectively.

[0009] The sugar contained in the second sugar liquid must be sugar which does not produce a crystal substantially [in liquid nitrogen temperature, do not carry out phase separation, and] like mannite (mannitol) or an inulin. If a crystal is not produced substantially, it means not producing the crystal of extent which does damage to the structure and bioactive of an organization after preservation. As for this sugar, what does not need to permeate intracellular and cannot permeate intracellular easily rather is desirable.

[0010] As for the first sugar liquid, it is desirable that sugar which is useful to maintenance of the bioactive of an organization, for example, a glucose, is included. As for the concentration of a glucose, it is desirable to consider as the nearest possible concentration equally to the glucose concentration under organization. The first sugar liquid needs to consider as the presentation which does not cause hemolysis constituting the cause of a residual of the corpuscle component of a under [an organization]. If hemolysis is not produced substantially, it means that there are not structure of the organization by hemolysis and damage on bioactive.

[0011] Although it is desirable that it is an isotonicity (isotonic) to an organization substantially as for the first and second sugar liquid, what is necessary is just 1/2 of blood or lymph thru/or the osmotic pressure of the twice as many range as this. The first and second sugar liquid may also contain salts besides sugar, an acid, a base, a buffer, a surfactant, a thickener, etc., respectively. As for the first [which is used for perfusion], and second temperature of sugar liquid, it is desirable to maintain at as low the temperature more than the freezing point of liquid (usually -10 degrees C thru/or +5 degrees C) as possible in order to prevent change of an organization.

[0012] Mixed liquor with water must not carry out phase separation of the organic solvent used for the second sugar liquid in liquid nitrogen temperature, and it must not produce a crystal substantially. If a crystal is not produced substantially, it means not producing the crystal of extent which does damage to the structure and bioactive of an organization after preservation. Organic solvents equipped with such a property are a glycerol and dimethyl sulfoxide. Although 5 - 10% of the concentration of the organic solvent in the second sugar liquid is suitable, ** is also highly (for example, 15%) good a little lower (for example, 3%) a little than this.

[0013] As for the metal needle and metal plinth which are used for the needle aggregate,

what consists of the metal from which a front face does not affect an organization and honeydew, and produces neither rust nor corrosion by them at least is desirable. For example, stainless steel, gold, platinum, gilding, chrome plating (iron, brass, etc.), etc. are used.

[0014] The size of a metal needle needs to be taken as the size which gives sufficient mechanical strength to run through with an organization sample. Heat conduction is so prompt that spacing is so narrow that a metal needle is thick, and although the temperature of an organization sample can be quickly raised in case preservation at low temperature is ended, the mechanical damage of the whole organization sample becomes large. The size of a metal needle is 0.2. There is nothing and 1.6 mm and spacing (center to center) are 0.5. Or 4mm is suitable.

[0015] As for cooling of the organization sample with which the metal needle was run through, it is desirable to perform even -80 degrees C gradually at least, for example, it is desirable to consider a temperature fall as the average of less than 1.3 degrees C/m to -80 degrees C. Such a temperature fall stores a sample in the heat insulation box of suitable magnitude, and if it places all over a -80-degree C freezer, it is realizable. The freezer by which program control was carried out may be used.

[0016] In case preservation is ended, by making quickly a part of [at least] temperature of the needle aggregate into 20 degrees C or more within for example, 2 minutes, to the temperature more than the melting point of the second sugar liquid (usually - 5 degrees C thru/or +5 degrees C, for example, 4 degrees C), the temperature of a sample is raised quickly and thawed. Specifically, a suitable quantity of tepid water (20 degrees C thru/or 40 degrees C) (liquids other than water are sufficient) is poured around a metal plinth. Or the perfusate (sugar liquid) of the suitable temperature for the surroundings of the sample run through by the metal needle aggregate is passed. By such approach, the temperature of an organization sample is preferably raised within 4 minutes more than the freezing point of the second sugar liquid, and is thawed.

[0017] After a sample dissolves, an organization sample is drawn out from the needle aggregate by drawing out the cloth and synthetic paper with which are a suitable means, for example, the base of a metal needle was run through. Perfusion of the drawn-out organization sample is carried out by suitable temperature, for example, a 4-degree C physiological saline, sugar liquid, tissue culture liquid, and others, it recovers the bioactive needed at least and the business after it is presented with it.

[0018] Carrying out perfusion with the liquid (for example, the same liquid as the first sugar liquid) of a suitable presentation, in order to recover bioactive, temperature is raised over the time amount more than the minimum, and it is made to go up to near 37 more degree C from temperature required for defrosting of an organization to about 25 degrees C. The minimum time amount is 5 minutes and it is desirable to raise temperature to 25 degrees C over 10 minutes or more. There is no limit of time amount especially in the rise to near 37 degree C from the temperature of 25 degrees C. dibutyryl cyclicAMP may also be included in the perfusate used in this phase, and it is useful to prevention of the cell membrane damage under hypoxia concentration. hydrocortizone may also be included in perfusate again.

[0019] Especially this invention is useful to cold storage, such as the liver of mammalian, the kidney, the pancreas, a spleen, a testis, the ovary, a suprarenal gland, a brain, and the thyroid. It is effective for especially the cold storage of liver.

[0020]
[Function] The first sugar liquid is injected into the blood vessel which passes to the organ of an animal, and the blood in an organ is eliminated by permuting blood with the first sugar liquid. By injecting the second sugar liquid into a blood vessel furthermore, and permuting the first sugar liquid with the second sugar liquid, the first sugar liquid is eliminated and the inside of a blood vessel is filled with the second sugar liquid. A sinusoid and an intercellular space are also filled with the second sugar liquid at this time.
[0021] If the needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through with the organization sample excised (a perfusion front stirrup may be excised in perfusion) and it cools gradually, the temperature of the whole organization sample will be comparatively maintained at homogeneity by heat conduction through the run-through metal needle. The organization sample run through by the needle aggregate is saved at liquid nitrogen temperature by reducing temperature further to liquid nitrogen temperature, and maintaining at this temperature.
[0022] Since the second sugar liquid introduced into the organization from the blood vessel contains the sugar which does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], the crystal of sugar itself and ice does not produce it at liquid nitrogen temperature at least besides the cell of an organization (a sinusoid and an intercellular space are included in a blood vessel). Moreover, it mixes with the second sugar liquid with water well, and mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and it contains the organic solvent which does not produce a crystal substantially. This organic solvent permeates intracellular. Since this organic solvent exists, the inside and outside of a cell do not produce an ice or solvent (or eutectic object) crystal during an organization in liquid nitrogen temperature, either. So, destruction of the cell by generation of ice and a solvent crystal is prevented.
[0023] If a part of [at least] temperature of the needle aggregate is quickly made into 20 degrees C or more in case preservation is ended, the temperature of an organization sample will rise to more than the melting point of the second sugar liquid, and if the part which touches a metal needle dissolves, a sample can be drawn out from the needle aggregate. If the temperature of an organization is furthermore raised at 25 degrees C or more, an organization will recover the bioactive. By heat conduction through the run-through metal needle aggregate, the temperature of the whole organization sample rises to homogeneity promptly and comparatively compared with the case where the metal needle aggregate is not used. For this rapid and uniform temperature rise of the whole sample, the property and function of a tissue cell are not spoiled and adjustment of subsequent environments (temperature etc.) can recover them.
[0024]
[Example] An example is shown below and it considers as still more concrete explanation of this invention.
[Example 1]
(1) a with a manufacture outer-diameter die length [of the metal needle aggregate / 15mm die length of 0.3mm] stainless steel hypodermic needle (what excluded the connection section to a glass syringe) -- the plinth of low melting alloys -- 1mm spacing -- length -- width 12 train erection was carried out ten train (a tip -- facing up). In order to

take out the inserted organization sample, the glass rod of 0.6mm of sizes was inserted in the shape of a grid between needles along with the plinth.
[0025] (2) liver perfusion -- the laparotomy was performed on the healthy rat and the detention needle was inserted in the hepatic portal vein. According to the conventional method of liver perfusion, by the flow rate of 4ml/m, the first perfusate of the following presentation made into the temperature of 4 degrees C was poured into the hepatic portal vein, and carried out perfusion for 5 minutes from the polyethylene capillary connected with the detention needle.
D-mannitol 1 gKrebs-Ringer buffer solution 20ml, subsequently, by the flow rate of 4ml/m, the second perfusate of the following presentation made into the temperature of 4 degrees C was poured into the hepatic portal vein, and carried out perfusion for 5 minutes.
Dimethyl sulfoxide 2gD-mannite 1 gKrebs-Ringer buffer solution 20ml [0026] (3) Liver was extracted immediately after cold storage perfusion, and the metal needle aggregate was run through. After storing this in the form polystyrene heat insulation box, it stored all over the -80-degree C freezer (slowly cooled at less than 1 degree C/m). The heat insulation box was filled with the liquid nitrogen of optimum dose after 3-hour or more progress, and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.
[0027] (4) The wave plate was fixed to the location with a depth [of a constant temperature bath with a defrosting temperature / of an organization / of 25 degrees C] of 2 millimeters. The liver sample which it let pass to the metal needle was taken out from the heat insulation box as it was, and it carried on this wave plate. the 25-degree C constant temperature to which a metal plinth flows around -- it is heated with water. It took out after 2 minutes, the liver sample was extruded using the grid of the glass rod inserted in the plinth side of a metal needle, and it removed from the needle. Perfusion of the first perfusate with a temperature of 4 degrees C was poured in and carried out to liver for 5 minutes by the flow rate of 4ml/m from the portal vein the bottom picking outside, and the second perfusate in liver was permuted by the first perfusate.
[0028] (5) After carrying out formalin fixation of the check liver sample of organization preservation, it dyed by making it an intercept with a thickness of 5 microns. As a result of microscope observation, the cell of liver is saved well and especially the glycogen granule was accepted clearly.
[0029] [Example 2]
(1) organization extraction and perfusion -- the abdominal cavity of a healthy rat was injected with 0.1ml of chloro bromazine 1% water solutions, and the weight of 100g, the abdominal peripheral nerve was intercepted, after injecting with and anesthetizing 0.04mg [per weight of 100g] SOMUNO pentyl, the laparotomy was performed and the detention needle connected with the polyethylene capillary was inserted in the hepatic portal vein. According to the conventional method of liver perfusion, it is per minute about the first perfusate of the following presentation which made temperature -5 degrees C from the polyethylene capillary connected with the detention needle. By the flow rate of 0.9ml, it poured into the hepatic portal vein and perfusion was carried out for 4 minutes. while carrying out perfusion -- liver -- the law from a rat -- it excised by the method.
D-glucose 2 gBSA 4.5 g enzyme inhibitor Pefabloc (Merck) 1 mM equivalent p-APMSF

0.1 mM equivalent (p-amidinophenylmethanesulfonylfluoride hydrochloride) Krebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) Chloro bromazine 1 g heparin 0.5 Milliliter [0030] While being immersed into the liquid (the second perfusate) of the following presentation which made extracted liver immediately the temperature of -5 degrees C, it is per minute about the second perfusate. By the flow rate of 0.9ml, it poured into the hepatic portal vein and perfusion was carried out for 4 minutes. Dimethyl sulfoxide 10gD-mannite 5gD-glucose 2 gBSA 4.5g enzyme inhibitor Pefabloc (Merck) 1 mM equivalent p-APMSF It is Krebs-Henseleit by 0.1mM. Buffer solution 100 Milliliter (pH 7.4) [0031] (2) The metal needle aggregate (diameter of a gilding brass needle and a needle 5mm in 0.8mm, main spacing of 3mm, height of 20mm of a needle, thickness of a metal plinth) was run through with liver immediately after cold storage perfusion. The base of a metal needle is beforehand stabbed with the synthetic paper. This was enclosed with the cold-resistance polyethylene bag, and it cooled to -80 degrees C all over the program control freezer set as -1 degree C per minute. After 3-hour or more progress, in the heat insulation box, the liquid nitrogen of optimum dose was poured out and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.

[0032] (3) After taking out the container of a sample out of the defrosting liquid nitrogen of an organization and placing for a while all over a -80-degree C freezer, in the case of 50g organization weight, the temperature of the whole sample was raised to -4 degrees C in a short time for less than 2 minutes by circulating liquid in the defrosting liquid of the same presentation as the first perfusate.

[0033] The sample was sampled from the metal needle aggregate in defrosting liquid, perfusion of the first perfusate with a temperature of -4 degrees C was poured in and carried out from the detention needle for 4 minutes by the flow rate of 1ml/m to the portal vein, and the second perfusate in liver was permuted by the first perfusate.

[0034] When some organizations were fixed and dyed with the conventional method and optical microscope observation was carried out, most change of an organization was not seen as compared with the organization immediately after excision (only perfusion by the first perfusate).

[0035] [Example 3]

(1) preparation of perfusion -- the abdominal cavity of a healthy rat was injected with the 0.1ml [per weight of 100g] chlorpromazine 1% water solution, the abdominal peripheral nerve was intercepted, per ml, after carrying out 50 microliter injection per weight of 100g, halothane anesthesia of the heparin liquid of 1000IU was carried out with the conventional method, the laparotomy was performed, and the detention needle connected with the polyethylene capillary was inserted in the hepatic portal vein (portal vein cannulation). Cannulation was performed also to the bottom main artery by the same approach (bottom main artery cannulation), and the bottom main artery and the inferior vena cava were litigated collectively. The main artery was stopped by the crane mel, the thorax was opened and a main artery and vena cava were cut.

[0036] (2) Cut the ligation section of a perfusion inferior vena cava, lead bottom main artery cannulation in the first perfusate of the following presentation which made temperature -5 degrees C, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it poured into the hepatic portal vein, respectively, and perfusion was carried out for 10

minutes. After checking that blood had been enough eliminated from liver, liver was extracted from the rat. At this time, a portal vein and a bottom main artery secure each cannulation to a liver side, and are cut.
The first perfusate: D-glucose 2 gBSA 3 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) Chlorpromazine 0.01g [0037] While being immersed into the liquid (the second perfusate) of the following presentation which made extracted liver immediately the temperature of -5 degrees C, bottom main artery cannulation is led in this second perfusate, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it poured into the hepatic portal vein, respectively, and perfusion was carried out for 10 minutes.
Dimethyl sulfoxide 10 gD-mannite 5 gBSA 3 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) [0038] (2) The metal needle aggregate (diameter of a gilding brass needle and a needle 5mm in 0.8mm, main spacing of 3mm, height of 20mm of a needle, thickness of a metal plinth) was run through with liver immediately after cold storage perfusion. The base of a metal needle is beforehand stabbed with the synthetic paper. This was enclosed with the cold-resistance polyethylene bag, and it cooled to -80 degrees C all over the program control freezer set as -1 degree C per minute. After 3-hour or more progress, in the heat insulation box, the liquid nitrogen of optimum dose was poured out and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.
[0039] (3) After taking out the container of a sample out of the defrosting liquid nitrogen of an organization and placing for a while all over a -80-degree C freezer, the organization sample was dipped in the defrosting liquid (temperature of about 30 degrees C) of the same presentation as the first perfusate, and, in the case of 50g organization weight, the temperature of the whole sample was raised to 4 degrees C in a short time for less than 2 minutes by circulating liquid.
[0033] A sample is sampled from the metal needle aggregate in defrosting liquid, bottom main artery cannulation is led in the third perfusate of the following presentation, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it poured into the hepatic portal vein, respectively, perfusion was carried out for 10 minutes, and the second perfusate in liver was permuted by the third perfusate. Temperature of the third perfusate was made into 4 degrees C at first, and raised temperature to 25 degrees C at a rate of about 2.5 degrees C per minute in perfusion.
The third perfusate: D-mannitol 1 gD-glucose 1 g lactobionate 50 mMBSA 3 g ascorbic acid 0.1 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) [0040] When some organizations were fixed and dyed with the conventional method and optical microscope observation was carried out, most change of an organization was not seen as compared with the organization immediately after excision (only perfusion by the first perfusate).
[0041] They are the temperature of 37 degrees C, and relative humidity about the liver which finished perfusion by the third perfusate in the example 3. It stores into 100% of box, and is [2-14C] of 185kBq. Diazepam was poured in from portal vein cannulation and the perfusate for a metabolic turnover experiment of the following presentation was supplied by the flow rate of 1 ml/min using the immediately after metering pump. This liquid was supplied to coincidence by the flow rate of 1.5 ml/min through artery cannulation. Perfusion was continued for 20 minutes, the perfusate which flows out from

liver at this time was received in ROUTO, and it extracted in the test tube every 2 minutes.
[0042] The extracted perfusate carried out at-long-intervals alignment separation by 3000 rotations for 10 minutes, separated the radioactive metabolite in the supernatant liquid by thin-layer chromatography, and performed detection analysis by radio RUMINOGURAFI. Consequently, 4'-hydroxydiazepam and Nordazepam which are the metabolite besides Diazepam which is a parent compound in effluent, Temazepam, and Oxazepam It was detected. These are metabolite accepted when the same experiment is conducted using the liver which does not carry out cryopreservation, and show that the liver tissue saved by this approach has metabolic activity.
[0043] Moreover, any effluent is abbreviation when the quantum of the lactate dehydrogenase (LDH) was carried out. It is 100IU / liter and normal values were shown through the perfusion for 20 minutes. This shows that liver tissue has not received damage even if it passes through the process of freezing and defrosting.
[0044]
[Effect of the Invention] As for living thing organizations saved by this invention, such as an organ and muscles, the property and function of the cell are often maintained. Especially the approach of this invention is effective in preservation of the tissue of liver and the kidney.

[Industrial Application] This invention relates to the cold storage approach and the preservation playback approach of the living thing organization which can maintain the property and function of a cell which constitute a living thing organization store method, especially an organization.

[Description of the Prior Art] Since cells, such as microorganisms, such as bacteria, and an ovum, a sperm, a cultured cell, are saved so that activity may be maintained 50% or more, a cell is gradually cooled with the speed around 1 degree C/m, it saves around -200 degrees C, and the approach of dissolving quickly (defrosting) is learned at the time of preservation termination. Under the present circumstances, in order to prevent destruction by freezing of a cell, a cell is placed into 10% glycerol or 5 - 10% dimethyl sulfoxide water solution.

[Effect of the Invention] As for living thing organizations saved by this invention, such as an organ and muscles, the property and function of the cell are often maintained. Especially the approach of this invention is effective in preservation of the tissue of liver and the kidney.

[Problem(s) to be Solved by the Invention] The cell according to individuals, such as a microorganism, an ovum, and a sperm, can be comparatively saved by the above-mentioned approach for a long period of time. However, when such liquid was injected into organs, such as the organ of an animal, for example, liver, and the kidney, the property of the cell of an organization and the loss of a function which were saved were remarkable.

[0004] The purpose of this invention is offering the approach of saving the organization which constitutes the organ of a living thing so that the property's and function's may be maintained on cell level. Moreover, the purpose of this invention is offering the approach of recovering bioactive, after saving the organization which constitutes the organ of a living thing so that the property and function may be maintained on cell level.

[Means for Solving the Problem] In order that the living thing organization store method of this invention may attain this purpose, the first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to the predetermined organ of an animal. The sugar which permutes the blood in an organ with the first sugar liquid, and does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is injected into a blood vessel. The second sugar liquid permutes the first sugar liquid in a blood vessel, and all or the predetermined part of organs is excised. The needle aggregate which consists of the metal needle of a large number fixed in parallel is run through with the sample of this excised organ. In case cool gradually at the rate of predetermined, temperature is further reduced to liquid nitrogen temperature, the sample with which the needle aggregate was run through is saved at liquid nitrogen temperature and preservation is ended, a part of [at least] temperature of the needle aggregate is quickly made into 20 degrees C or more. The temperature of a sample is promptly raised to more than the freezing point of the second sugar liquid, and it consists of drawing out a sample from the needle aggregate. This invention is applicable not only to organs, such as an organ of an animal, but connective tissue, such as muscles.

[0006] Instead of excising an organization sample after a permutation (henceforth perfusion) with the first [of blood], and second sugar liquid, all or the predetermined part of organs may be excised from an animal between the perfusion before perfusion. A predetermined part is one of the hepatic lobes in liver, or two. In order to perform perfusion or excision, it is necessary to conduct an operation for an animal. When performing perfusion after excision, some organizations may be further excised as a sample after perfusion.

[0007] Before undergoing an operation, it is desirable to intercept the peripheral nerve near [used as especially an object] the organ of an animal because of maintenance of an organization function. Cutoff of the peripheral nerve near an object organ can be attained by pouring into the inside of a blood vessel, or intraperitoneal the matter which has

monoamine oxidase (following, MAO, and brief sketch) inhibitory action. There are for example, N-amino alkyl phenothiazin system compound, N-amino alkyl JIBENZOAZA cycloheptadiene system compound, an isonicotinic acid hydrazide system compound, and a propargyl amine derivative in MAO inhibitor applicable to this invention. For example, they are chlorpromazine, a promethazine, imipramine, the iproniazid, pargyline, etc. MAO inhibitor may be added also in perfusate.

[0008] Perfusion usually stabs with a detention needle (hollow) the blood vessel which passes to an organ, and is performed. A flow rate is chosen according to the class of organ, the class of blood vessel, and a size. The permutation with the first and second sugar liquid is enough performed so that blood and the first sugar liquid may not remain substantially, respectively.

[0009] The sugar contained in the second sugar liquid must be sugar which does not produce a crystal substantially [in liquid nitrogen temperature, do not carry out phase separation, and] like mannite (mannitol) or an inulin. If a crystal is not produced substantially, it means not producing the crystal of extent which does damage to the structure and bioactive of an organization after preservation. As for this sugar, what does not need to permeate intracellular and cannot permeate intracellular easily rather is desirable.

[0010] As for the first sugar liquid, it is desirable that sugar which is useful to maintenance of the bioactive of an organization, for example, a glucose, is included. As for the concentration of a glucose, it is desirable to consider as the nearest possible concentration equally to the glucose concentration under organization. The first sugar liquid needs to consider as the presentation which does not cause hemolysis constituting the cause of a residual of the corpuscle component of a under [an organization]. If hemolysis is not produced substantially, it means that there are not structure of the organization by hemolysis and damage on bioactive.

[0011] Although it is desirable that it is an isotonicity (isotonic) to an organization substantially as for the first and second sugar liquid, what is necessary is just 1/2 of blood or lymph thru/or the osmotic pressure of the twice as many range as this. The first and second sugar liquid may also contain salts besides sugar, an acid, a base, a buffer, a surfactant, a thickener, etc., respectively. As for the first [which is used for perfusion], and second temperature of sugar liquid, it is desirable to maintain at as low the temperature more than the freezing point of liquid (usually -10 degrees C thru/or +5 degrees C) as possible in order to prevent change of an organization.

[0012] Mixed liquor with water must not carry out phase separation of the organic solvent used for the second sugar liquid in liquid nitrogen temperature, and it must not produce a crystal substantially. If a crystal is not produced substantially, it means not producing the crystal of extent which does damage to the structure and bioactive of an organization after preservation. Organic solvents equipped with such a property are a glycerol and dimethyl sulfoxide. Although 5 - 10% of the concentration of the organic solvent in the second sugar liquid is suitable, ** is also highly (for example, 15%) good a little lower (for example, 3%) a little than this.

[0013] As for the metal needle and metal plinth which are used for the needle aggregate, what consists of the metal from which a front face does not affect an organization and honeydew, and produces neither rust nor corrosion by them at least is desirable. For example, stainless steel, gold, platinum, gilding, chrome plating (iron, brass, etc.), etc. are

used.
[0014] The size of a metal needle needs to be taken as the size which gives sufficient mechanical strength to run through with an organization sample. Heat conduction is so prompt that spacing is so narrow that a metal needle is thick, and although the temperature of an organization sample can be quickly raised in case preservation at low temperature is ended, the mechanical damage of the whole organization sample becomes large. The size of a metal needle is 0.2. There is nothing and 1.6 mm and spacing (center to center) are 0.5. Or 4mm is suitable.
[0015] As for cooling of the organization sample with which the metal needle was run through, it is desirable to perform even -80 degrees C gradually at least, for example, it is desirable to consider a temperature fall as the average of less than 1.3 degrees C/m to -80 degrees C. Such a temperature fall stores a sample in the heat insulation box of suitable magnitude, and if it places all over a -80-degree C freezer, it is realizable. The freezer by which program control was carried out may be used.
[0016] In case preservation is ended, by making quickly a part of [at least] temperature of the needle aggregate into 20 degrees C or more within for example, 2 minutes, to the temperature more than the melting point of the second sugar liquid (usually - 5 degrees C thru/or +5 degrees C, for example, 4 degrees C), the temperature of a sample is raised quickly and thawed. Specifically, a suitable quantity of tepid water (20 degrees C thru/or 40 degrees C) (liquids other than water are sufficient) is poured around a metal plinth. Or the perfusate (sugar liquid) of the suitable temperature for the surroundings of the sample run through by the metal needle aggregate is passed. By such approach, the temperature of an organization sample is preferably raised within 4 minutes more than the freezing point of the second sugar liquid, and is thawed.
[0017] After a sample dissolves, an organization sample is drawn out from the needle aggregate by drawing out the cloth and synthetic paper with which are a suitable means, for example, the base of a metal needle was run through. Perfusion of the drawn-out organization sample is carried out by suitable temperature, for example, a 4-degree C physiological saline, sugar liquid, tissue culture liquid, and others, it recovers the bioactive needed at least and the business after it is presented with it.
[0018] Carrying out perfusion with the liquid (for example, the same liquid as the first sugar liquid) of a suitable presentation, in order to recover bioactive, temperature is raised over the time amount more than the minimum, and it is made to go up to near 37 more degree C from temperature required for defrosting of an organization to about 25 degrees C. The minimum time amount is 5 minutes and it is desirable to raise temperature to 25 degrees C over 10 minutes or more. There is no limit of time amount especially in the rise to near 37 degree C from the temperature of 25 degrees C. dibutyryl cyclicAMP may also be included in the perfusate used in this phase, and it is useful to prevention of the cell membrane damage under hypoxia concentration. hydrocortizone may also be included in perfusate again.
[0019] Especially this invention is useful to cold storage, such as the liver of mammalian, the kidney, the pancreas, a spleen, a testis, the ovary, a suprarenal gland, a brain, and the thyroid. It is effective for especially the cold storage of liver.

[Function] The first sugar liquid is injected into the blood vessel which passes to the organ of an animal, and the blood in an organ is eliminated by permuting blood with the first sugar liquid. By injecting the second sugar liquid into a blood vessel furthermore, and permuting the first sugar liquid with the second sugar liquid, the first sugar liquid is eliminated and the inside of a blood vessel is filled with the second sugar liquid. A sinusoid and an intercellular space are also filled with the second sugar liquid at this time.
[0021] If the needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through with the organization sample excised (a perfusion front stirrup may be excised in perfusion) and it cools gradually, the temperature of the whole organization sample will be comparatively maintained at homogeneity by heat conduction through the run-through metal needle. The organization sample run through by the needle aggregate is saved at liquid nitrogen temperature by reducing temperature further to liquid nitrogen temperature, and maintaining at this temperature.
[0022] Since the second sugar liquid introduced into the organization from the blood vessel contains the sugar which does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], the crystal of sugar itself and ice does not produce it at liquid nitrogen temperature at least besides the cell of an organization (a sinusoid and an intercellular space are included in a blood vessel). Moreover, it mixes with the second sugar liquid with water well, and mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and it contains the organic solvent which does not produce a crystal substantially. This organic solvent permeates intracellular. Since this organic solvent exists, the inside and outside of a cell do not produce an ice or solvent (or eutectic object) crystal during an organization in liquid nitrogen temperature, either. So, destruction of the cell by generation of ice and a solvent crystal is prevented.
[0023] If a part of [at least] temperature of the needle aggregate is quickly made into 20 degrees C or more in case preservation is ended, the temperature of an organization sample will rise to more than the melting point of the second sugar liquid, and if the part which touches a metal needle dissolves, a sample can be drawn out from the needle aggregate. If the temperature of an organization is furthermore raised at 25 degrees C or more, an organization will recover the bioactive. By heat conduction through the run-through metal needle aggregate, the temperature of the whole organization sample rises to homogeneity promptly and comparatively compared with the case where the metal needle aggregate is not used. For this rapid and uniform temperature rise of the whole sample, the property and function of a tissue cell are not spoiled and adjustment of subsequent environments (temperature etc.) can recover them.

[Example] An example is shown below and it considers as still more concrete explanation of this invention.
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(1) a with a manufacture outer-diameter die length [of the metal needle aggregate / 15mm die length of 0.3mm] stainless steel hypodermic needle (what excluded the connection section to a glass syringe) -- the plinth of low melting alloys -- 1mm spacing -

- length -- width 12 train erection was carried out ten train (a tip -- facing up). In order to take out the inserted organization sample, the glass rod of 0.6mm of sizes was inserted in the shape of a grid between needles along with the plinth.
[0025] (2) liver perfusion -- the laparotomy was performed on the healthy rat and the detention needle was inserted in the hepatic portal vein. According to the conventional method of liver perfusion, by the flow rate of 4ml/m, the first perfusate of the following presentation made into the temperature of 4 degrees C was poured into the hepatic portal vein, and carried out perfusion for 5 minutes from the polyethylene capillary connected with the detention needle.
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Dimethyl sulfoxide 2gD-mannite 1 gKrebs-Ringer buffer solution 20ml [0026] (3) Liver was extracted immediately after cold storage perfusion, and the metal needle aggregate was run through. After storing this in the form polystyrene heat insulation box, it stored all over the -80-degree C freezer (slowly cooled at less than 1 degree C/m). The heat insulation box was filled with the liquid nitrogen of optimum dose after 3-hour or more progress, and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.
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(1) organization extraction and perfusion -- the abdominal cavity of a healthy rat was injected with 0.1ml of chloro bromazine 1% water solutions, and the weight of 100g, the abdominal peripheral nerve was intercepted, after injecting with and anesthetizing 0.04mg [per weight of 100g] SOMUNO pentyl, the laparotomy was performed and the detention needle connected with the polyethylene capillary was inserted in the hepatic portal vein. According to the conventional method of liver perfusion, it is per minute about the first perfusate of the following presentation which made temperature -5 degrees C from the polyethylene capillary connected with the detention needle. By the flow rate of 0.9ml, it poured into the hepatic portal vein and perfusion was carried out for 4 minutes. while carrying out perfusion -- liver -- the law from a rat -- it excised by the method.

D-glucose 2 gBSA 4.5 g enzyme inhibitor Pefabloc (Merck) 1 mM equivalent p-APMSF 0.1 mM equivalent (p-amidinophenylmethanesulfonylfluoride hydrochloride) Krebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) Chloro bromazine 1 g heparin 0.5 Milliliter [0030] While being immersed into the liquid (the second perfusate) of the following presentation which made extracted liver immediately the temperature of -5 degrees C, it is per minute about the second perfusate. By the flow rate of 0.9ml, it poured into the hepatic portal vein and perfusion was carried out for 4 minutes. Dimethyl sulfoxide 10gD-mannite 5gD-glucose 2 gBSA 4.5g enzyme inhibitor Pefabloc (Merck) 1 mM equivalent p-APMSF It is Krebs-Henseleit by 0.1mM. Buffer solution 100 Milliliter (pH 7.4) [0031] (2) The metal needle aggregate (diameter of a gilding brass needle and a needle 5mm in 0.8mm, main spacing of 3mm, height of 20mm of a needle, thickness of a metal plinth) was run through with liver immediately after cold storage perfusion. The base of a metal needle is beforehand stabbed with the synthetic paper. This was enclosed with the cold-resistance polyethylene bag, and it cooled to -80 degrees C all over the program control freezer set as -1 degree C per minute. After 3-hour or more progress, in the heat insulation box, the liquid nitrogen of optimum dose was poured out and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.

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[0033] The sample was sampled from the metal needle aggregate in defrosting liquid, perfusion of the first perfusate with a temperature of -4 degrees C was poured in and carried out from the detention needle for 4 minutes by the flow rate of 1ml/m to the portal vein, and the second perfusate in liver was permuted by the first perfusate.

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(1) preparation of perfusion -- the abdominal cavity of a healthy rat was injected with the 0.1ml [per weight of 100g] chlorpromazine 1% water solution, the abdominal peripheral nerve was intercepted, per ml, after carrying out 50 microliter injection per weight of 100g, halothane anesthesia of the heparin liquid of 1000IU was carried out with the conventional method, the laparotomy was performed, and the detention needle connected with the polyethylene capillary was inserted in the hepatic portal vein (portal vein cannulation). Cannulation was performed also to the bottom main artery by the same approach (bottom main artery cannulation), and the bottom main artery and the inferior vena cava were litigated collectively. The main artery was stopped by the crane mel, the thorax was opened and a main artery and vena cava were cut.

[0036] (2) Cut the ligation section of a perfusion inferior vena cava, lead bottom main artery cannulation in the first perfusate of the following presentation which made temperature -5 degrees C, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it

poured into the hepatic portal vein, respectively, and perfusion was carried out for 10 minutes. After checking that blood had been enough eliminated from liver, liver was extracted from the rat. At this time, a portal vein and a bottom main artery secure each cannulation to a liver side, and are cut.
The first perfusate: D-glucose 2 gBSA 3 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) Chlorpromazine 0.01g [0037] While being immersed into the liquid (the second perfusate) of the following presentation which made extracted liver immediately the temperature of -5 degrees C, bottom main artery cannulation is led in this second perfusate, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it poured into the hepatic portal vein, respectively, and perfusion was carried out for 10 minutes.
Dimethyl sulfoxide 10 gD-mannite 5 gBSA 3 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) [0038] (2) The metal needle aggregate (diameter of a gilding brass needle and a needle 5mm in 0.8mm, main spacing of 3mm, height of 20mm of a needle, thickness of a metal plinth) was run through with liver immediately after cold storage perfusion. The base of a metal needle is beforehand stabbed with the synthetic paper. This was enclosed with the cold-resistance polyethylene bag, and it cooled to -80 degrees C all over the program control freezer set as -1 degree C per minute. After 3-hour or more progress, in the heat insulation box, the liquid nitrogen of optimum dose was poured out and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.
[0039] (3) After taking out the container of a sample out of the defrosting liquid nitrogen of an organization and placing for a while all over a -80-degree C freezer, the organization sample was dipped in the defrosting liquid (temperature of about 30 degrees C) of the same presentation as the first perfusate, and, in the case of 50g organization weight, the temperature of the whole sample was raised to 4 degrees C in a short time for less than 2 minutes by circulating liquid.
[0033] A sample is sampled from the metal needle aggregate in defrosting liquid, bottom main artery cannulation is led in the third perfusate of the following presentation, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it poured into the hepatic portal vein, respectively, perfusion was carried out for 10 minutes, and the second perfusate in liver was permuted by the third perfusate. Temperature of the third perfusate was made into 4 degrees C at first, and raised temperature to 25 degrees C at a rate of about 2.5 degrees C per minute in perfusion.
The third perfusate: D-mannitol 1 gD-glucose 1 g lactobionate 50 mMBSA 3 g ascorbic acid 0.1 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) [0040] When some organizations were fixed and dyed with the conventional method and optical microscope observation was carried out, most change of an organization was not seen as compared with the organization immediately after excision (only perfusion by the first perfusate).
[0041] They are the temperature of 37 degrees C, and relative humidity about the liver which finished perfusion by the third perfusate in the example 3. It stores into 100% of box, and is [2-14C] of 185kBq. Diazepam was poured in from portal vein cannulation and the perfusate for a metabolic turnover experiment of the following presentation was supplied by the flow rate of 1 ml/min using the immediately after metering pump. This liquid was supplied to coincidence by the flow rate of 1.5 ml/min through artery

cannulation. Perfusion was continued for 20 minutes, the perfusate which flows out from liver at this time was received in ROUTO, and it extracted in the test tube every 2 minutes.

[0042] The extracted perfusate carried out at-long-intervals alignment separation by 3000 rotations for 10 minutes, separated the radioactive metabolite in the supernatant liquid by thin-layer chromatography, and performed detection analysis by radio RUMINOGURAFI. Consequently, 4'-hydroxydiazepam and Nordazepam which are the metabolite besides Diazepam which is a parent compound in effluent, Temazepam, and Oxazepam It was detected. These are metabolite accepted when the same experiment is conducted using the liver which does not carry out cryopreservation, and show that the liver tissue saved by this approach has metabolic activity.

[0043] Moreover, any effluent is abbreviation when the quantum of the lactate dehydrogenase (LDH) was carried out. It is 100IU / liter and normal values were shown through the perfusion for 20 minutes. This shows that liver tissue has not received damage even if it passes through the process of freezing and defrosting.